EPIMYSIAL CONNECTIVE TISSUE POLYSACCHARIDES OF BOVINE SEMIMEMBRANOSUS MUSCLE AND ALTERATIONS IN THEIR TYPE WITH AGE AND SEX DIFFERENCES

SUMMARY—Newborn veal, 11.5-month-old steers, 14.5-month-old heifers and 9 to 10.5-year-old cows were used to identify the polysaccharide types present in the epimysium and to determine the relationship between the amount of connective tissue polysaccharides and the amount of collagen in the epimysium. Trimmed muscle was used for tenderness evaluation by shear force. Average amounts of 332, 247, 230 and 202 mg dry polysaccharides per 100g dry, defatted epimysium were isolated from the veal, steer, heifer and cow groups, respectively. A negative correlation was obtained between age of animals and amount of isolated polysaccharides. Only 20% of the hexosamine in the dried defatted epimysium was extracted as soluble connective tissue polysaccharides. It is possible that some selective solubilization of certain polysaccharides occurred during extraction and, consequently, the extracted polysaccharides may not reflect the actual polysaccharide composition of the intact tissue. Considering this, it was found by using Dowex 1 X-2 chromatography that an average of 42% of the total uronic acids of the veal and heifer groups was eluted with 0.5M NaCl. 17 and 19% of the cow and steer uronic acids, respectively, were eluted with 0.5M NaCl. In the veal and heifer groups, 37 and 38% of the total uronic acids were eluted with 1.5M NaCl, whereas in the cow and steer groups the amount represented 71 and 70%. The percentages of uronic acids eluted with 2.0M NaCl were 20, 11, 19 and 12% in the veal, steer, heifer and cow groups, respectively. Dermatan sulfate was found to be the main polysaccharide eluted with 1.5M NaCl for the veal, heifers and cows. It also represented an important type in the steers, although other sulfate polysaccharides seemed to be present. Cellulose polyacetate electrophoresis confirmed that hyaluronic acid and dermatan sulfate were present in the epimysium. The ratio of hexosamine to insoluble collagen in the epimysium was positively associated with muscle tenderness.

INTRODUCTION

IT IS generally accepted that collagen affects the eating qualities of meat. In muscle and in other tissues, collagen occurs in close association with connective tissue polysaccharides (Clayson et al., 1962; Meyer, 1965). Specific connective tissue polysaccharide types tend to occur along tightly packed collagen fibers; others are associated with loosely organized collagen fibers (Meyer, 1965). So far, eight different types of connective tissue polysaccharides have been isolated from a variety of tissues such as cartilage, bone, tendon, skin and vascular tissue (Meyer, 1957; Walker, 1961). These are hyaluronic acid, chondroitin, chondroitin sulfate A, chondroitan sulfate C, dermatan sulfate, heparin, heparitin sulfate and keratosulfate.

It has been observed that transformations take place in the connective tissue polysaccharides of several organs or structures during aging (Milch, 1966; Jackson and Bentley, 1968). Some researchers found that total connective tissue polysaccharides, as measured by hexosamine, decreased during aging (Houck and Jacob, 1958; Sobel et al., 1954; Shetlar and Masters, 1955). Other researchers pointed out that there were some alterations in polysaccharide types during aging (Loewi and Meyer, 1958). McIntosh (1967) suggested that during the postmortem aging of meat, degradation of connective tissue polysaccharides occurs, resembling the type of breakdown caused by papain. Fox (1968) found that in three muscles of bulls, steers and cows, the hexosamine content of the polysaccharides eluted with 0.5, 1.25, 1.5 and 2.0M NaCl did not vary significantly between sexes or time post-mortem. No significant association was found between hexosamine content of muscle and tenderness as measured by shear force. No significant relationships were found between mucopolysaccharide fractions and tough or tender meat. Wipf et al. (1970) observed that porcine muscle classified as pale, soft and exudative had a higher hexosamine content, and higher dermatan sulfate, residual acid mucopolysaccharide and chondroitin content than normal muscle. Tenderness of porcine muscle was positively correlated with dermatan sulfate content.

Additional research is needed to establish more definitely the type of connective tissue polysaccharides in bovine muscle. The present study was undertaken to isolate the polysaccharides of bovine epimysium, to fractionate and further characterize them and to determine if alterations in polysaccharide types occurred with age and sex differences.

MATERIALS & METHODS

Experimental animals

3 female veal (2 to 3 days of age), 3 steers (11.5 months), 3 heifers (14.5 months) and 3 cows (9 to 10.5 years) were used to study the age and sex differences of muscle connective tissue polysaccharides. The study was designed mainly to study intensively the connective tissue polysaccharides of animals varying widely in age. Thus, the number of experimental animals per age group was kept small. Steers were included in the experimental group, since they constitute a large proportion of the animals slaughtered for meat. All experimental animals were of the Holstein breed. The veal, steers and heifers were obtained from sources where actual birth records were kept, while the age of the last group was estimated by a veterinarian. Carcasses averaged 27 kg for the veal, 275 kg for the steers, 125 kg for the heifers and 273 kg for the cows. All animals were slaughtered at the Cornell Department of Animal Science Meat Abattoir according to practices normally employed at the laboratory.

Processing of muscle

Within 30 min after death, the right and left semimembranosus muscles were removed and frozen in large polyethylene bags at −29°C. The frozen storage period varied from 4–6 months. At the convenient time, the muscles were allowed to thaw and age in a 2°C cooler for 8 days. At the end of the aging period, the muscles were removed from the polyethylene bags, the right muscle utilized for the chemical determinations and the left muscle immediately cooked and used for tenderness evaluation.

Characterization of connective tissue polysaccharides

This study was conducted on the epimysial sheath of the semimembranosus muscle because it represented a concentrated source of connective tissue. The epimysium of the right semimembranosus muscle was carefully dissected with a scalpel, cut into pieces and extracted with a 0.6M KCl buffer at 2°C during 3 hr according to the method of McIntosh (1961). Solvent-to-tissue ratios of 20:1 (v/w) were used. Following this extraction, the epimysium was defatted in 10 vol of acetone at 2°C for 12 hr with occasional stirring. The acetone was changed and the same procedure was repeated. The defatted epimysium was dried to constant weight in a vacuum desiccator, powdered in a Wiley mill and stored in a desiccator at −29°C. Triplicate 30-mg samples of dry defatted epimysium were assayed for hexosamine as described subsequently, to calculate recoveries after the isolation procedure.

To isolate the polysaccharides of the epimysium, 10g of dried defatted epimysium was digested with papain according to a modification of the original procedure of Schiller et al. (1961). Major changes in the Schiller procedure consisted of increasing the amount of papain from 2 to 25 mg per g of dried defatted material, extending the 0.5N NaOH treatment from 4 to 24 hr and omitting the trypsin digestion. Type II crude papain from Sigma Chemical Company, St. Louis, Missouri, was purified according to the method of Kimmel and Smith

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Table 1—Dry weight, hexosamine and hexuronic acid content of polysaccharides isolated from bovine epimysium.

Animal group	Animal no.	Dry weight of polysaccharide (mg/100g dry epimysium)	Hexosamine (μg/mg dry polysaccharide)	Hexuronic[a] acid (μg/mg dry polysaccharide)	Hexosamine: hexuronic acid ratio[b] Dry epimysium	Hexosamine: hexuronic acid ratio[b] Extracted polysaccharide
Veal	1	290	206	213	2.41	1.05
	2	385	234	239	2.59	1.11
	3	289	206	197	2.50	1.13
Heifer	1	233	228	210	3.13	1.18
	2	218	223	214	3.37	1.13
	3	240	199	201	3.10	1.07
Cow	1	195	197	183	2.77	1.17
	2	217	199	173	3.25	1.25
	3	193	212	197	4.28	1.15
Steer	1	236	219	197	3.69	1.20
	2	275	225	227	3.57	1.07
	3	231	203	186	3.68	1.18

[a]Hexuronic acid by carbazole procedure (Dische, 1947).
[b]Molar ratio.

Table 2—Elution pattern of polysaccharides rechromatographed on cellulose columns, expressed in terms of percent hexosamine eluted at each ethanol concentration.

Percent ethanol in elution solvent[a]	Veal 1	Veal 3	Heifer 1	Heifer 2	Heifer 3	Cow 3	Steer 1
	(%)						
80	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0
30	0	7.8	0	5.0	0	0	7.1
25	0	0	0	0	0	0	4.0
20	0	5.6	0	0	5.3	0	5.5
15	6.5	0	21.6	0	0	0	3.9
10	13.3	8.4	3.7	8.9	0	9.1	0
5	13.6	17.7	14.1	27.9	35.2	5.5	20.8
0	66.6	60.5	60.6	58.2	59.5	85.4	58.7
0[b]	0	0	0	0	0	0	0

[a]8-ml fractions were collected at each ethanol concentration.
[b]An additional tube was collected with 0% ethanol to ensure complete elution of the polysaccharides.

(1954) and utilized for the digestion. The polysaccharides were isolated as a cetylpyridinium chloride complex, redissolved and precipitated with ethanol. The purified polysaccharides were washed with ethanol and ether, dried in vacuo over P_2O_5 and the dry weight of the isolated polysaccharides recorded. They were analyzed for hexosamine or hexuronic acid, and utilized for anionic exchange chromatography and electrophoresis.

The colorimetric analysis for hexosamine was carried out as described by Boas (1953). The hydrolysis conditions were modified according to the procedure of Anastassiadis and Common (1958) to ensure maximum recovery of the hexosamine. Balazs et al. (1965) reported that when the color intensity produced by the Boas procedure was measured at 530 mμ, the amount of color produced by glucosamine was different from the amount of color produced by galactosamine. To determine the relative amounts of glucosamine and galactosamine in the isolated polysaccharides, therefore, their absorption spectra were recorded between 580 and 510 mμ and compared to a standard curve prepared with known ratios of glucosamine to galactosamine. A Beckman Model DU 2 recording spectrophotometer was used for these measurements. The colorimetric carbazole method of Dische (1947) was used for the determination of hexuronic acids. In this method, iduronic acid, which is the uronic acid moiety of dermatan sulfate, gives less color than does glucuronic acid. However, there is another method of uronic acid determination based on an orcinol reaction (Brown, 1946), in which both iduronic acid and glucuronic acid give the same color yield. This method was used when an attempt was made to establish the type of polysaccharide present in the epimysium.

For the purpose of fractionating the epimysial polysaccharides, chromatography on Dowex 1 ×-2 chloride columns was conducted according to the method of Schiller et al. (1961). The polysaccharides were theoretically expected to be fractionated in the following manner: hyaluronic acid is eluted with 0.5M NaCl; heparitin sulfate, chondroitin, chondroitin sulfates A and C and dermatan sulfate are eluted with 1.5M NaCl; heparin and part of keratosulfate are eluted with 2.0M NaCl (it requires 3.0M NaCl to obtain a satisfactory elution of keratosulfate). The effluent was collected in 8-ml fractions with an automatic fraction collector and analyzed for hexuronic acid (Dische, 1947). To obtain the resolution of the polysaccharide mixture eluted with 1.5M NaCl, the effluent was pooled, dialyzed until free of chloride ions, concentrated and refractionated on a cellulose column by means of ethanol gradient solution. The method used in this fractionation was that of Gardell (1957), which elutes the polysaccharides as follows: chondroitin sulfate C at 40–50% ethanol (Meyer et al., 1956); chondroitin sulfate A at 30–40% ethanol (Meyer et al., 1956); heparin sulfate at 10% ethanol (Gardell, 1957). In preliminary work for this research, pure chondroitin sulfate A was nearly completely eluted with 30% ethanol and dermatan sulfate was eluted between 15 and 0% ethanol (Cormier, 1969). The effluent was collected in 8-ml fractions and analyzed for hexosamine.

Cellulose polyacetate electrophoresis was used to separate the polysaccharides isolated from the epimysium of the semimembranosus muscle of the four groups of animals. The separation was conducted according to the method of Mathews (1961). Cellulose polyacetate strips—Seraphore III—were soaked in pyridine formic acid buffer at pH 3, the samples were spotted along with known purified standards (generously furnished by Dr. J. A. Cifonelli, University of Chicago). A voltage of 170 was applied for 45 min. The strips were dried, sprayed with 1% acridine orange, washed and dried in a fume hood.

Analysis of epimysial collagen

Since it has been shown that collagen and polysaccharides interact under physiological conditions (Mathews, 1965), it was felt that a better understanding of meat connective tissue as a whole would be obtained if the solubility characteristics of epimysial collagen were studied concomitantly with the characterization of the connective tissue polysaccharides. Duplicate 200-mg samples of dry defatted epimysium were analyzed for soluble and insoluble collagen. The alkali-soluble fraction was obtained following the procedure of Kao and McGavack (1959). The insoluble collagen fraction was extracted by the method of Fitch et al. (1955). The two collagen fractions were analyzed for hydroxyproline content using the method of Prockop and Udenfriend (1960). Collagen content was calculated by multiplying the amount of hydroxyproline by 7.52 (Goll et al., 1963).

Tenderness evaluation

Clayson et al. (1962) found there was some degree of uniformity in the proportion of collagen and polysaccharides present in the epimysium compared to the intramuscular connective tissue. This uniformity was deemed to justify a comparison between the amount of connective

Table 3—Optical densities at 570 and 530 mμ of glucosamine and galactosamine standards and hexosamines from the epimysial polysaccharides.

Origin of hexosamine		Optical density (OD) 570 mμ	Optical density (OD) 530 mμ	Glucosamine: galactosamine ratio
Hexosamine standards				
50 μg glu: 0 μg gal		.111	.414	1.00:0
40 μg glu:10 μg gal		.116	.406	0.80:0.20
30 μg glu:20 μg gal		.134	.420	0.60:0.40
20 μg glu:30 μg gal		.143	.425	0.40:0.60
10 μg glu:40 μg gal		.145	.408	0.20:0.80
0 μg glu:50 μg gal		.149	.392	0:1.00
Isolated epimysial polysaccharides				
Animal no.	μg[a]			
Veal 1	20.3	.102	.305	0.41:0.59[b]
Veal 2	20.4	.122	.379	0.52:0.48
Veal 3	8.3	.068	.205	0.43:0.57
Heifer 1	19.2	.113	.347	0.48:0.52
Heifer 2	20.0	.119	.366	0.49:0.51
Heifer 3	19.6	.094	.297	0.57:0.43
Cow 1	19.9	.111	.305	0.14:0.86
Cow 2	19.5	.114	.319	0.20:0.80
Cow 3	No sample left			
Steer 1	5.5	.055	.167	0.45:0.55
Steer 2	20.0	.131	.398	0.45:0.55
Steer 3	No sample left			

[a]Weight of dry isolated polysaccharides giving the intensity of absorption obtained during the scanning procedure.

[b]Values obtained from a standard curve constructed of the ratio $\frac{\text{OD at 570 m}\mu}{\text{OD at 530 m}\mu}$ for the pure hexosamine standards.

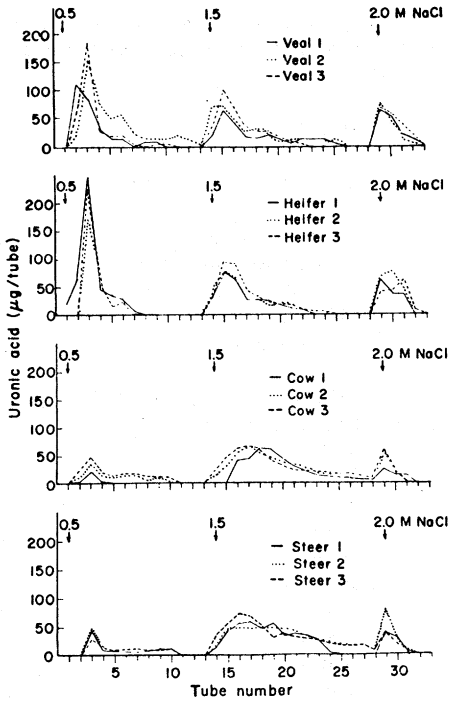

Fig. 1—Dowex 1 x-2 chloride column chromatography of 5 mg of polysaccharides from veal, heifer, cow and steer using NaCl of increasing molarity as the eluant. 8-ml fractions were collected in an automatic fraction collector.

tissue polysaccharides present in the epimysium and the tenderness attributes of the trimmed semimembranosus muscle. To conduct the tenderness evaluation, four slices, each 3.8 cm thick, were cut from the middle section of the left semimembranosus muscle, perpendicular to the length of the muscle. The slices were placed into deep beef fat previously heated to 150°C and cooked to an internal temperature of 63°C. The meat was allowed to cool at 25°C and 8 cores, each having a diameter of 1.27 cm, were mechanically removed from each slice. Each core was sheared in half on a Warner-Bratzler shearing apparatus. An average of 32 shear readings was recorded as the shear value for that muscle.

Data analysis

The data were analyzed statistically by analysis of variance according to Steel and Torrie (1960). Non-orthogonal single degree of freedom contrasts were used to determine significance of mean differences. Simple correlations as outlined by Steel and Torrie were used to determine relationships between the variables under study.

RESULTS & DISCUSSION

Isolation of the polysaccharides

Table 1 presents a summary of the quantity of polysaccharides isolated from the epimysium along with an analysis of their hexosamine and hexuronic acid content. There were fewer polysaccharides isolated with increasing animal age. A correlation of -0.63, $P < .05$, was obtained between quantity of polysaccharides and animal age. This relationship must be interpreted with caution, however, since the isolated polysaccharides contained only approximately 20% of the total hexosamine originally present in the dry defatted epimysium. This low percentage recovery can be attributed to the difficulty of working quantitatively during the numerous steps of the isolation procedure and to the interference of hexosamine containing mucoproteins in the pre-isolation hexosamine measurement. Table 1 presents the molar ratios of hexosamine to hexuronic acid in the pre-isolation and post-isolation material. The pre-isolation hexosamine:hexuronic acid ratios ranged from 2.41 to 4.28, with an average ratio of 3.19. The average post-isolation ratio came down to 1.14 and there were no statistically significant differences between the animal groups. These results can probably be explained by the incomplete removal of hexosamine containing mucoproteins from the epimysium during the pre-isolation treatment with a 0.6M KCl buffer. Boas (1955) found that almost 50% of the hexosamine present in rat connective tissue was from plasma mucoprotein, whereas the other 50% was from the connective tissue polysaccharides.

In the present research, the pieces that the epimysium was cut into for the KCl buffer extraction were not very small. A more effective removal of mucoproteins would have been accomplished if the epimysium had been pulverized before the extraction procedure. When pre- and post-isolation hexuronic acid measurements were used to calculate percent recovery during the polysaccharide isolation procedure, an average recovery of 49% was obtained. There were no significant differences between animal groups for uronic acid recovery. It cannot be ruled out that selective losses of polysaccharides did not occur during the isolation procedure. Further experimentation is needed to clarify this point. The hexosamine content of the isolated polysaccharides ranged from 197–234 μg per mg of isolated polysaccharides; the hexuronic acid content as measured by the carbazole method ranged from 173–228 μg per mg of isolated polysaccharides. An analysis of variance revealed no statistically significant differences between the bovine groups for the hexosamine or the hexuronic acid content of the isolated polysaccharides.

Chromatography of the isolated polysaccharides on Dowex 1 x-2 chloride columns

Results obtained with Dowex 1 x-2 chloride chromatography are summarized in Figure 1. The data for the four groups—veal, heifer, cow and steer—are graphed separately to emphasize the similarities and differences observed between these groups. The veal and heifer groups had similar polysaccharide peak intensities at 0.5, 1.5 and 2.0M NaCl; the cow

Table 4—Uronic acid values obtained by two different colorimetric procedures for polysaccharide standards, polysaccharides eluted with 0.5M NaCl and polysaccharides rechromatographed on cellulose column.

Origin of material	Carbazole procedure (μg/ml)	Orcinol procedure (μg/ml)	Carbazole:orcinol ratio
A. Polysaccharide standards[a]			
Chondroitin sulfate A	59.8	29.4	2.03
Chondroitin sulfate C	–	–	2.0–2.5[b]
Dermatan sulfate	21.2	67.7	0.31
Heparin	85.6	22.8	3.75
Hyaluronic Acid	60.9	40.0	1.52
B. Epimysial polysaccharides eluted with 0.5M NaCl (hyaluronic acid fraction)[c]			
Veal 1	6.2	3.5	1.77
Veal 2	10.2	6.4	1.61
Veal 3	8.8	5.8	1.51
Heifer 1	11.8	8.5	1.39
Heifer 2	11.1	7.2	1.54
Heifer 3	15.7	9.9	1.59
Cow 1	4.6	2.5	1.79
Cow 2	5.7	4.1	1.37
Cow 3	3.6	3.1	1.18
Steer 1	6.6	4.1	1.61
Steer 2	8.2	5.0	1.65
Steer 3	5.2	3.4	1.55
C. Epimysial rechromatographed polysaccharides on a cellulose column[d]			
Veal 1	1.9	4.5	0.42
Veal 3	1.9	5.6	0.34
Heifer 1	1.9	3.8	0.51
Heifer 2	1.1	3.5	0.30
Heifer 3	1.7	6.6	0.26
Cow 3	2.2	6.0	0.37
Steer	2.2	6.1	0.36

[a]Purified chondroitin sulfate A, dermatan sulfate, hyaluronic acid and heparin were gifts of Dr. J. A. Cifonelli from the University of Chicago. They were rechromatographed on cellulose columns and the material assayed came from the peak of the elution curve at the proper ethanol concentration. The heparin was used without further purification.

[b]Personal communication with Dr. J. A. Cifonelli.

[c]The material assayed came from the peak of the elution curve with 0.5M NaCl during Dowex 1 X-2 chromatography.

[d]The material assayed came from the peak of the elution curve, which occurred at 0% ethanol. The five missing values were rejected because of imperfections in the cellulose columns.

and steer groups also had similar peak intensities at the three NaCl concentration levels but had a pattern strikingly different from the veal and heifer groups. An analysis of variance of the percentage of uronic acid eluted at the three levels of NaCl concentration indicated that at the 0.5M NaCl level (hyaluronic acid) there was a statistically significant difference, $P < .01$, between the veal and the heifer groups compared to the cow and the steer groups. These same comparisons were also significantly different with the 1.5M NaCl eluant, $P < .001$, and with the 2.0M NaCl eluant, $P < .05$. An average of 42% of the total uronic acids was eluted as hyaluronic acid in the veal group compared to 17% in the aged cow group. This observation was in general agreement with previous findings made with pig skin (Loewi and Meyer, 1958) and bovine vitreous humor (Chvapil, 1967), and indicated that the level of hyaluronic acid tended to decrease with aging. The relative proportion of hyaluronic acid, 42% of the total uronic acids, was exactly the same in the veal and heifer groups. This observation was unexpected in light of the references just cited. The percentage of uronic acids eluted as hyaluronic acid in the steer group was 19, a value close to the 17 obtained for the aged cow group. Asboe-Hansen (1963) reported that the female hormone estrogen increased the polysaccharide content of connective tissue and, more specifically, the hyaluronic acid content. The reduced level of estrogens in the aged cows and the absence of estrogen and testosterone in the castrated steers conceivably could explain the low hyaluronic acid content observed in these two groups of animals.

Refraction of polysaccharides eluted with 1.5M NaCl

The polysaccharides eluted with 1.5M NaCl were dialyzed, concentrated, precipitated on top of cellulose columns and then gradually redissolved by means of a decreasing ethanol gradient. Table 2 presents the results obtained. The data from five columns had to be rejected either because of an overflow of the eluting solvent on top of the column or because of air pockets developing in the column. The average recovery for the seven samples appearing in Table 2 was 89%.

Of the seven samples rechromatographed, most of the polysaccharides were eluted with an ethanol concentration of 15% or less. The elution pattern of the epimysial polysaccharides closely followed the pattern of a pure dermatan standard run along with the isolated polysaccharides. Traces of chondroitin sulfate A seemed to be present in veal No. 3, heifer No. 2 and steer No. 1. According to these limited observations, no change in sulfated polysaccharide type could be detected among bovine epimysia of different ages.

Electrophoresis of isolated epimysial polysaccharides

The isolated polysaccharides were resolved into two or three components by electrophoresis on cellulose polyacetate at pH 3; typical results of one member of each group are presented in Figure 2. The veal contained two spots, one running parallel to hyaluronic acid and the other parallel to dermatan sulfate. The heifer contained two similar spots plus traces of an additional one running parallel to chondroitin sulfate A. The cow had only traces of hyaluronic acid and a major spot running parallel to dermatan sulfate. The steer had one spot running parallel to hyaluronic acid and a major component running parallel to dermatan sulfate. The information obtained from the fractionation of epimysial polysaccharides with electrophoresis confirmed results previously reported for the Dowex 1 x-2 chloride chromatography. In both procedures, the veal and heifer were found to have a sizable amount of hyaluronic acid whereas the cow and steer had a smaller amount. The electrophoretic result also supported the findings obtained with the cellulose column fractionations, which indicated that dermatan sulfate was the major sulfated polysaccharide in bovine epimysium.

Absorption spectrum of hexosamines in isolated polysaccharides

Table 3 presents the optical density at 570 and 530 mμ of pure hexosamine standards and of the hexosamine obtained from the isolated polysaccharides. The veal, heifer and steer groups had glucosamine:galactosamine ratios ranging

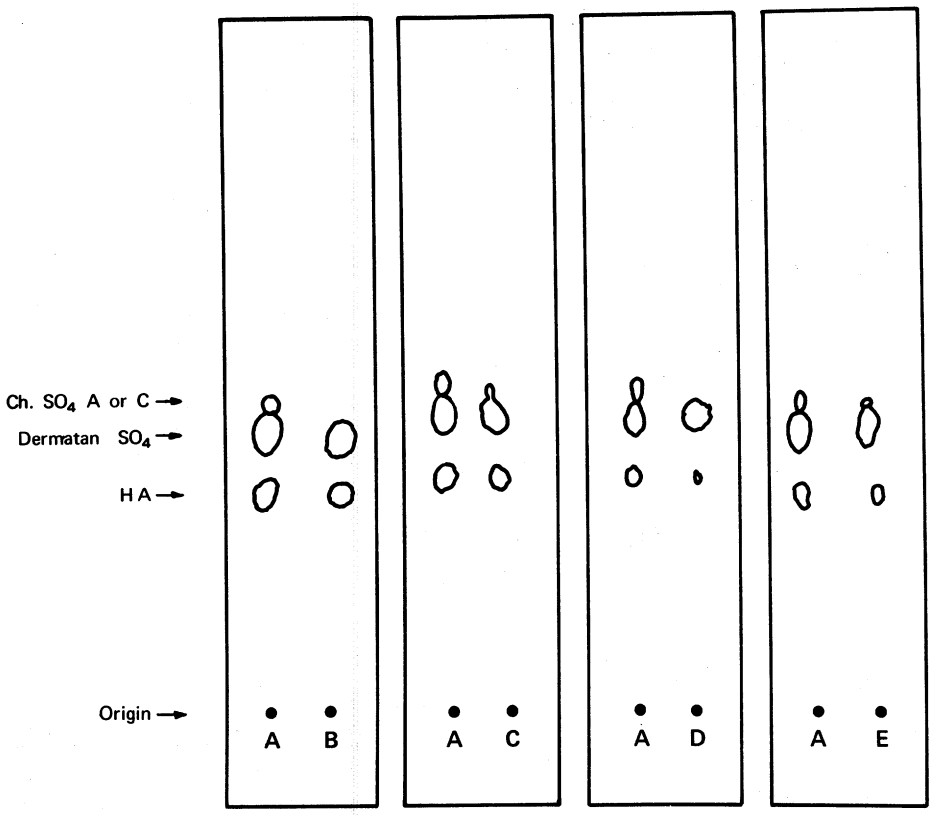

Fig. 2—Cellulose polyacetate electrophoresis in pyridine formic acid buffer pH 3 of the polysaccharides isolated from bovine epimysium and of polysaccharide standards. At point A, three polysaccharide standards were spotted: hyaluronic acid (HA), dermatan sulfate and chondroitin sulfate A (Ch SO_4 A). The veal polysaccharides were spotted at point B, the heifer at point C, the cow at point D and the steer at point E.

Table 5—Collagen fractions of semimembranosus epimysium and hexosamine:collagen interrelationship.

Animal group	Animal no.	Alkali-soluble collagen	Insoluble collagen	Hexosamine	Hexosamine:insoluble collagen ratio
			(g/100g dry epimysium)		
Veal	1	0.99	40.59	60	1.47
	2	1.03	39.68	90	2.27
	3	1.01	42.36	67	1.57
Heifer	1	0.27	32.71	53	1.62
	2	0.40	37.94	49	1.28
	3	0.43	31.82	48	1.50
Cow	1	0.40	35.31	38	1.09
	2	0.26	37.88	43	1.14
	3	0.50	34.90	41	1.17
Steer	1	0.90	39.60	52	1.31
	2	0.62	42.94	62	1.44
	3	1.23	43.55	50	1.13

from 0.41:0.59 to 0.57:0.43. These ratios indicated that 41 to 57% of the hexosamines in these three groups were glucosamine. The cow group had a glucosamine:galactosamine ratio, indicating that 80–86% of the hexosamines were galactosamine. Assuming that the glucosamine in the veal and heifer groups comes from hyaluronic acid, the ratios appearing in Table 3 show that 40% or more of the total polysaccharides of these groups is accounted for by hyaluronic acid; the cow group shows 14–20% glucosamine and thus 14–20% hyaluronic acid. In the steer group, approximately 45% of the hexosamines was glucosamine. In the Dowex chromatography, 20% of the uronic acids was eluted as hyaluronic acid. The remaining 25% of the hexosamine could arise from heparitin sulfate, keratosulfate and heparin, which also have glucosamine as their hexosamine moiety.

Identity of the hexuronic acids

In another attempt to identify the polysaccharide types, advantage was taken of the different behavior of uronic acid in the carbazole and orcinol procedures. Table 4 presents the results obtained by these two colorimetric methods for a) polysaccharide standards, b) the 0.5M NaCl fraction from Dowex chromatography (hyaluronic acid), and c) the polysaccharides rechromatographed on cellulose columns.

Results obtained from analysis of the 0.5M NaCl fraction of the Dowex columns confirmed that hyaluronic acid was definitely the polysaccharide eluted at this NaCl concentration, since the carbazole:orcinol ratios for all animals were close to that of the hyaluronic acid standard. Results obtained with the rechromatographed polysaccharides on cellulose columns confirmed that dermatan sulfate was the polysaccharide eluted near 0% during the ethanol fractionation.

Epimysial collagen

Table 5 presents the values obtained for the proportion of collagen which was alkali-soluble and alkali-insoluble. The quantity of collagen which could be solubilized in 0.1N NaOH varied among the animals: the highest amount was found in the veal group, the second highest in the steer group, the cow group came next and, finally, the heifer group had the least alkali-soluble collagen. An analysis of variance revealed that the veal group had significantly more alkali-soluble collagen than the steer group, $P < .10$ and the cow and heifer groups, $P < .01$; the steer group had significantly more alkali-soluble collagen than the heifer and the cow groups, $P < .01$. There were statistically significant differences in total collagen between the veal group and the heifer group, $P < .01$; between the veal group and the cow group, $P < .01$; between the steer group and the heifer group, $P < .001$ and between the steer and the cow group, $P < .01$. The highest amount of collagen observed in the veal compared to the cow group was in agreement with Goll et al. (1963) and Carmichael and Lawrie (1967). The lower collagen values observed in heifers No. 1 and No. 3 cannot be fully explained. Polysaccharides and collagen are thought to be intimately related in connective tissue (Meyer, 1965). In the present

study, a relationship was found between the hexosamine content of the isolated polysaccharides and the amount of insoluble collagen in the epimysium. These data appear in Table 5, presenting the amount of hexosamines in the isolated polysaccharides along with the hexosamine:insoluble collagen ratio. There was a significant negative correlation, −.45 and $P < .10$, between the amount of isolated epimysial polysaccharides as measured by hexosamine content and the amount of insoluble epimysial collagen.

Meat tenderness

Fields and Pearson (1969) reported that the collagen solubility pattern in the epimysium was closely related to the pattern found in intramuscular connective tissue. This observation justifies the comparison of findings made on the epimysium and the tenderness attributes of the whole semimembranosus muscle. In the present study, meat tenderness as measured by the Warner-Bratzler Shear varied significantly with the age of the animals. The tenderness data are summarized in Table 6. An analysis of variance indicated that the veal muscle was more tender than the heifer muscle, $P < .05$, and the cow muscle, $P < .001$. The steer muscle was more tender than the cow muscle, $P < .001$. There was no significant difference in tenderness between the veal and the steer muscle. A correlation of 0.85, $P < .001$, was obtained between the age of the animals and the shear force values. The more epimysial alkali-soluble collagen, the lower were the shear force values of the muscle. A negative correlation of −.64, $P < .05$, was obtained between tenderness as measured by shear force and percent alkali-soluble collagen in the epimysium. This finding suggests that the degree of solubility of the collagen should be considered when biochemical explanations for toughness are sought. There was also a significant association between the hexosamine:collagen ratio and the tenderness of the muscle as measured by shear force. The higher the hexosamine:collagen ratio, the lower the shear force value of the muscle. The correlation coefficient between these two measurements was 0.54, $P < .05$. No significant correlations were found between shear value and the polysaccharide fractions obtained with Dowex chromatography.

It would appear that the decrease of polysaccharides with advancing age may be a predominant factor affecting the increased insolubility of collagen with aging. When there are fewer polysaccharides forming a network around collagen fibers, there may be more chances for the formation of intramolecular cross linkages in collagen, which would decrease the solubility of collagen. The polysaccharides may play an important role in plasticizing the collagen fibers, and this may be how they contribute to meat tenderness, as has been suggested by Milch (1966).

Table 6—Tenderness of semimembranosus muscles of veal, heifer, cow and steer as determined by shear force.

Animal group	Animal no.	Number of replications	Mean shear force (lb)	Standard error
Veal	1	30	4.2	0.7
	2	30	4.2	0.5
	3	30	4.3	0.6
Heifer	1	32	11.3	1.0.
	2	32	7.4	1.3
	3	32	8.9	1.8
Cow	1	32	14.1	2.3
	2	32	14.5	2.1
	3	32	21.2	4.4
Steer	1	32	7.5	1.3
	2	32	7.5	1.2
	3	32	7.5	1.3

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